METHODS OF SCREENING FOR CONDENSATE-ASSOCIATED SPECIFICITY AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application No. 62/902,316, filed on Sep. 18, 2019, the content of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to the field of biological condensates.

BACKGROUND

[0003] Cells contain membrane-bound organelles such as mitochondria, lysosomes, and the endoplasmic reticulum, to, in part, localize various cellular functions. In addition to membrane-bound organelles, cells contain distinct sub-compartments that do not comprise a membrane between them and their immediate surrounding solution. Numerous of these membrane-less molecular assemblies have been shown to be formed through a process termed liquid-liquid phase separation or condensation, in a manner analogous to the partitioning of oil droplets in water. During this process, e.g., a solution comprising biological macromolecules separates into different phases, namely, a condensate dense phase that is enriched in at least some of the biological macromolecules and a surrounding light phase. A number of cellular condensates have been recognized that play important roles in biology (Banani et al., 2017, *Nat Rev Mol Cell Biol,* 18:285-298).

[0004] Various condensates are known to be important for modulating specific cellular processes in different cell types. Mechanistically, for example, a condensate can bring together molecules at an elevated concentration to accelerate reactions inside the condensate, or can sequester molecules in the condensate thereby reducing their concentration and preventing their activity in the surrounding medium. Aberrant condensate function has also been implicated in various human diseases, such as neurodegenerative, proliferative, immunological, cardiac, or metabolic disease (Naumann et al., 2018, *Nat Commun,* 9(1):335; Wegmann et al., 2018, *EMBO J,* 37(7): e98049; and Aguzzi et al., 2016, 26(7): 547-558). However, there is a lack of understanding of the mechanisms governing partitioning of a single macromolecule into or out of a condensate, and there is little or nothing known regarding whether a compound can selectively alter the partitioning of a single macromolecule into or out of a condensate.

[0005] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

BRIEF SUMMARY

[0006] Provided herein are methods of identifying a compound that preferentially affects a level of association of a first macromolecule with one or more target condensates, the method comprising: (a) contacting a cellular composition with a compound, wherein (i) the cellular composition comprises the one or more target condensates; and/or (ii) the one or more target condensates form simultaneously with and/or after contacting the cellular composition with the compound; and (b) determining the level of association of the first macromolecule with the one or more target condensates and a level of association of at least one additional macromolecule with the one or more target condensates, wherein the compound preferentially affects the level of association of the first macromolecule with the one or more target condensates if the compound alters the level of the first macromolecule as compared to a first reference level more than the compound alters the level of each additional macromolecule as compared to a reference level for each additional macromolecule. In some embodiments, the compound does not measurably alter the level of each additional macromolecule compared to the reference level for each additional macromolecule.

[0007] Also provided herein are methods of identifying a compound that preferentially increases a level of association of a first macromolecule with one or more target condensates, the method comprising: (a) contacting a cellular composition with a compound, wherein (i) the cellular composition comprises the one or more target condensates; and/or (ii) the one or more target condensates form simultaneously with and/or after contacting the cellular composition with the compound; and (b) determining the level of association of the first macromolecule with the one or more target condensates and a level of association of at least one additional macromolecule with the one or more target condensates, wherein the compound preferentially increases the level of association of the first macromolecule with the one or more target condensates if the compound increases the level of the first macromolecule as compared to a first reference level more than the compound increases the level of each additional macromolecule as compared to a reference level for each additional macromolecule. In some embodiments, the compound does not measurably increase the level of each additional macromolecule compared to the reference level for each additional macromolecule.

[0008] Also provided herein are methods of identifying a compound that preferentially decreases a level of association with one or more target condensates of a first macromolecule, the method comprising: (a) contacting a cellular composition with a compound, wherein (i) the cellular composition comprises the one or more target condensates; and/or (ii) the one or more target condensates form simultaneously with and/or after contacting the cellular composition with the compound; and (b) determining the level of association of the first macromolecule with the one or more target condensates and a level of association of at least one additional macromolecule with the one or more target condensates, wherein the compound preferentially decreases the level of association of the first macromolecule with the one or more target condensates if the compound alters the level of the first macromolecule as compared to a first reference level more than the compound decreases the level of each additional macromolecule as compared to a reference level for each additional macromolecule. In some embodiments, the compound does not measurably decrease the level of each additional macromolecule compared to the reference level for each additional macromolecule.

[0009] In some embodiments, the first reference level is a level of association of the first macromolecule with one or more reference condensates determined in the absence of the compound. In some embodiments, the reference level for each additional macromolecule is a level of association for